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UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
CEDAR 043453

Total Pages in this Submission
55

TO THE ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

METHOD FOR USING POTASSIUM CHANNEL AGONISTS FOR DELIVERING A MEDICANT TO AN ABNORMAL BRAIN REGION AND/OR A MALIGNANT TUMOR

and invented by:

Keith L. Black and Nagendra S. Ningaraj

If a CONTINUATION APPLICATION, *check appropriate box and supply the requisite information:*

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

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Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 34 pages and including the following:
   - a. ☒ Descriptive Title of the Invention
   - b. ☐ Cross References to Related Applications *(if applicable)*
   - c. ☐ Statement Regarding Federally-sponsored Research/Development *(if applicable)*
   - d. ☐ Reference to Microfiche Appendix *(if applicable)*
   - e. ☒ Background of the Invention
   - f. ☒ Brief Summary of the Invention
   - g. ☒ Brief Description of the Drawings *(if drawings filed)*
   - h. ☒ Detailed Description
   - i. ☒ Claim(s) as Classified Below
   - j. ☒ Abstract of the Disclosure

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Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*

   a. ☐ Formal b. ☒ Informal Number of Sheets 3

4. ☒ Oath or Declaration

   a. ☒ Newly executed *(original or copy)* ☐ Unexecuted

   b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*

   c. ☐ With Power of Attorney ☐ Without Power of Attorney

   d. ☐ *DELETION OF INVENTOR(S)*
   Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).

5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*
   The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche

7. ☐ Genetic Sequence Submission *(if applicable, all must be included)*

   a. ☐ Paper Copy

   b. ☐ Computer Readable Copy

   c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☒ Assignment Papers *(cover sheet & documents)*

9. ☒ 37 CFR 3.73(b) Statement *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations

12. ☐ Preliminary Amendment

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Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

16. ☒ Small Entity Statement(s) - Specify Number of Statements Submitted: 1

17. ☒ Additional Enclosures *(please identify below):*

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CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
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Indep. Claims	8	- 3 =	5	x $39.00	$195.00
Multiple Dependent Claims (check if applicable) ☐					$0.00
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Dated: **January 26, 2000**

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CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10) Applicant(s): **Keith L. Black and Nagendra S. Ningaraj**	Docket No. **CEDAR 043453**

Serial No.	Filing Date	Examiner	Group Art Unit
NOT ASSIGNED	**01/26/00**	--	--

Invention:

METHOD FOR USING POTASSIUM CHANNEL AGONISTS FOR DELIVERING A MEDICANT TO AN ABNORMAL BRAIN REGION AND/OR A MALIGNANT TUMOR

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VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27 (d)) - NONPROFIT ORGANIZATION

Docket No. CEDAR 043453

Serial No.	Filing Date	Patent No.	Issue Date
NOT ASSIGNED	HEREWITH	--	---

Applicant/ Patentee: **KEITH L. BLACK and NAGENDRA S. NINGARAJ**

Invention: **METHOD FOR USING POTASSIUM CHANNEL AGONISTS FOR DELIVERING A MEDICANT TO AN ABNORMAL BRAIN REGION AND/OR A MALIGNANT TUMOR**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: **Cedars-Sinai Medical Center**

ADDRESS OF ORGANIZATION: **8700 Beverly Boulevard**
Los Angeles, CA 90048

TYPE OF NONPROFIT ORGANIZATION:

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I hereby declare that the above-identified nonprofit organization qualifies as a nonprofit organization as defined in 37 C.F.R. 1.9(e) for purposes of paying reduced fees to the United States Patent and Trademark Office regarding the invention described in:

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TITLE IN ORGANIZATION: **SeniorVice-President for Legal Affairs and General Counsel**
ADDRESS OF PERSON SIGNING: **8700 Beverly Boulevard**
Los Angeles, CA 90048

SIGNATURE: _____ DATE: 1/24/00

APPLICATION

for

UNITED STATES LETTERS PATENT

on

METHOD FOR USING POTASSIUM CHANNEL AGONISTS FOR DELIVERING A MEDICANT TO AN ABNORMAL BRAIN REGION AND/OR A MALIGNANT TUMOR

by

Keith L. Black
and
Nagendra S. Ningaraj

CEDAR-0 43453
Sheets of Drawings: 3

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METHOD FOR USING POTASSIUM CHANNEL AGONISTS FOR DELIVERING A MEDICANT TO AN ABNORMAL BRAIN REGION AND/OR A MALIGNANT TUMOR

BACKGROUND OF THE INVENTION

Throughout the application various publications are referenced in parentheses. The disclosures of these publications in their entireties are hereby incorporated by reference in the application in order to more fully describe the state of the art to which this invention pertains.

1. THE FIELD OF THE INVENTION

This invention relates to the medical arts. In particular, it relates to a method of enhancing the delivery of a medicant across abnormal microvasculature to a tissue requiring treatment.

2. DISCUSSION OF THE RELATED ART

Pathologic neovascularization, i.e., the proliferation or development of new blood vessels, is essential for the growth and spread of primary, secondary and metastatic malignant tumors. It is known that certain properties of the new capillaries and arterioles constituting the neomicrovasculature in solid tumors differ from those of normal microvasculature. (J. Denekamp *et al.*, *Vasculature and microenvironmental gradients: the missing links in novel approaches to cancer therapy?*, Adv. Enzyme Regul. 38:281-99 [1998]). Neomicrovasculature induced by angiogenic factors from malignant cells was reported to possess altered pharmacological reactivity to some vasoconstricting agents, compared with neomicrovasculature that was not induced by neoplastic cells. (S.P. Andrade and W.T. Beraldo, *Pharmacological reactivity of neoplastic and non-neoplastic associated neovasculature to vasoconstrictors*, Int. J. Exp. Pathol. 79(6):425-32 [1998]).

A number of proposed cancer treatments have been based on differences between neomicrovasculature and normal microvasculature. For example, combretastatin A-4 was shown to cause vascular damage and occlusion selectively in the blood vessels of malignant tumors compared to normal blood vessels. (G.G. Dark *et al.*, *Combretastatin A-4, an agent that displays*

potent and selective toxicity toward tumor vasculature, Cancer Res. 57(10):1829-34 [1997]; D.J. Chaplin *et al.*, *Anti-vascular approaches to solid tumour therapy: evaluation of combretastatin A4 phosphate*, Anticancer Res. 19(1A):189-95 [1999]). Monoclonal antibodies have been directed to antigens and antigenic combinations specific to endothelial cells of pathologic neovasculature, such as vascular cell adhesion molecule (VCAM)-1, phosphatidylserine (PS), the glycoprotein endosialin, and prostate-specific membrane antigen (PSMA), with the aim of selectively inducing thrombosis in neovasculature. (E.g., S. Ran *et al.*, *Infarcation of solid Hodgkin's tumors in mice by antibody-directed targeting of tissue factor to tumor vasculature*, Cancer Res. 58(20):4646-53 [1998]; I. Ohizumi *et al.*, *Antibody-based therapy targeting tumor vascular endothelial cells suppresses solid tumor growth in rats*, Biochem. Biophys. Res. Commun. 236(2):493-96 [1997]; S.S. Chang *et al.*, *Five different antiprostate-specific membrane antigen (PSMA) antibodies confirm PSMA expression in tumor-associated neovasculature*, Cancer Res. 59(13):3192-98 [1999]; W.J. Rettig *et al.*, *Identification of endosialin, a cell surface glycoprotein of vascular endothelial cells in human cancer*, Proc. Natl. Acad. Sci. USA 89(22):10832-36 [1992]). But taken alone, shutting down blood flow through the neomicrovasculature to malignant tumors may not necessarily result in stopping tumor growth, because actively proliferating populations of neoplastic cells at the periphery of solid tumors may have access to blood supplied by normal microvasculature. (E.g., D.J. Chaplin *et al.* [1999]).

Consequently, other conventional and novel therapeutic modalities will continue to be of value in the treatment of malignant, solid tumors. However, the efficacy of novel therapeutic agents, including cytotoxic chemotherapeutic agents, monoclonal antibodies, cytokines, effector cells, and viral particles has been limited by their ability to reach their targets in vivo in adequate quantities. (E.g., R.K Jain, *Vascular and interstitial barriers to delivery of therapeutic agents in tumors*, Cancer Metastasis Rev. 9(3):253-66 [1990]). An important limiting factor is the low permeability to macromolecules and viral particles of neomicrovasculature supplying the tumors.

This problem of microvascular permeability is especially acute with respect to malignant tumors of the central nervous system. These malignancies are usually fatal, despite recent advances in the areas of neurosurgical techniques, chemotherapy and radiotherapy. In particular,

there are no standard therapeutic modalities that can substantially alter the prognosis for patients with malignant tumors of the brain, cranium, and spinal cord. For example, high mortality rates persist for patients diagnosed with malignant medulloblastomas, malignant meningiomas, malignant neurofibrosarcomas and malignant gliomas, which are characterized by infiltrative tumor cells throughout the brain. Although intracranial tumor masses can be debulked surgically, treated with palliative radiation therapy and chemotherapy, the survival associated with intracranial tumors, for example, a glioblastoma, is typically measured in months. The development of new therapeutic modalities against solid brain tumors largely depends on transvascular delivery of the potential therapeutic agent.

Transvascular delivery of chemotherapeutic agents and viral particles to tumor cells or other abnormal brain tissue is hampered by the blood-brain barrier, particularly the blood-tumor barrier found in brain tumors. The blood-brain barrier is a transvascular permeability barrier thought to result from the interendothelial tight junctions formed by the cerebrovascular endothelial cells of brain capillaries and arterioles in both normal and abnormal brain tissue. The blood-brain barrier protects the brain from changes in the composition of the systemic blood supply (e.g., in electrolytes) or from blood-borne macromolecules, such as immunoglobulins or other polypeptides, and prevents the transvascular delivery of many exogenously supplied pharmaceutical agents to brain tissues.

The treatment of brain tissue abnormalities, such as tumors, often involves the use of pharmaceutical agents with a significant toxicity of their own, making it highly desirable to be able to preferentially direct such agents to the abnormal or malignant tissue. While, there has been a great deal of interest in developing techniques which are capable of opening the blood-brain barrier to allow transport of pharmaceutical agents to the brain. Few of these methods are capable of selectively opening the blood-brain barrier only in the abnormal brain tissue while leaving the blood-brain barrier in the normal brain tissue intact.

For example, Neuwelt *et al.* used an intracarotid injection of hypertonic mannitol to osmotically disrupt the blood-brain barrier. They reported that this enhanced the uptake by brain tissue of inactivated HSV-1 particles that were administered immediately afterward by intracarotid bolus injection. (E.A. Neuwelt *et al.*, *Delivery of ultraviolet-inactivated 35S-*

herpesvirus across an osmotically modified blood-brain barrier, J. Neurosurg. 74(3):475-79 [1991]; Also, S.E. Doran *et al.*, *Gene expression from recombinant viral vectors in the central nervous system after blood-brain barrier disruption*, Neurosurgery 36(5):965-70 [1995]; G. Nilaver *et al.*, *Delivery of herpesvirus and adenovirus to nude rat intracerebral tumors after osmotic blood-brain barrier disruption*, Proc. Natl. Acad. Sci. USA 92(21):9829-33 [1995]).

Intracarotid infusion of leukotriene C.sub.4 (LTC.sub.4) selectively increases the permeability in brain tumor capillaries without affecting the permeability in normal brain capillaries. The effect of LTC.sub.4 on brain tumor capillaries is, however, limited to small molecules and it can only slightly increase the permeability of those small molecules in abnormal brain tissue relative to normal. Accordingly, LTC.sub.4 does not significantly increase the delivery of some larger water soluble molecules to brain tumors or other abnormalities.

The vasoactive nanopeptide bradykinin and agonists or polypeptide analogs thereof (e.g., receptor-mediated permeabilizers [RMPs]) have been injected intravenously to increase blood-brain barrier permeability to co-administered neuropharmaceutical or diagnostic agents. (B. Malfroy-Camine, *Method for increasing blood-brain barrier permeability by administering a bradykinin agonist of blood-brain barrier permeability*, U.S. Patent No. 5,112,596; J.W. Kozarich *et al.*, *Increasing blood brain barrier permeability with permeabilizer peptides*, U.S. Patent No. 5,268,164). Intracarotid infusion of bradykinin will selectively increase permeability 2- to 12-fold in brain tumor and ischemic brain capillaries for molecules ranging in molecular weight from 100 to 70,000 Daltons (Inamura, T. *et a l.*, Bradykinin selectively opens blood-tumor barrier in experimental brain tumors, J. Cereb. Blood Flow Metab. 14(5):862-70 [1994]). Bradykinin does not increase permeability in the normal blood brain barrier except at very high doses. (Wirth, K. *et al.*, *DesArg9-D-Arg[Hyp3,Thi5,D-Tic7,Oic8]bradykinin (desArg10-[Hoe140]) is a potent bradykinin B1 receptor antagonist*, Eur. J. Pharmacol. 205(2):217-18 [1991]). Opening of the blood-tumor barrier by bradykinin is transient, lasting 15 to 20 minutes. (Inamura *et al.* [1994]). After opening of abnormal brain capillaries with bradykinin, the capillaries become refractory to the bradykinin effect for up to 60 minutes. (Inamura *et al.* [1994]).

A method for selectively delivering to abnormal brain tissue a neuropharmaceutical agent (e.g., 5-fluorouracil, cisplatin, methotrexate, or monoclonal antibodies) or a diagnostic agent (e.g., technicium-99 glucoheptonate, gallium-EDTA, and ferrous magnetic or iodinated contrasting agents) employed intracarotid infusion of bradykinin, or a bradykinin analog, such as RMP-7; the bradykinin or bradykinin analog was administered approximately contemporaneously with the agent. (K.L. Black, *Method for selective opening of abnormal brain tissue capillaries*, U.S. Patent Nos. 5,527,778 and 5,434,137). Enhanced transvascular delivery of HSV-derived viral particles to malignant cells in the brains of rats was also achieved by disrupting the blood-brain barrier with bradykinin or RMP-7. (N.G. Rainov, *Selective uptake of viral and monocrystalline particles delivered intra-arterially to experimental brain neoplasms*, Hum. Gene. Ther. 6(12):1543-52 [1995]; N.G. Rainov *et al.*, *Long-term survival in a rodent brain tumor model by bradykinin-enhanced intra-arterial delivery of a therapeutic herpes simplex virus vector*, Cancer Gene Ther. 5(3):158-62 [1998]; F.H. Barnett *et al.*, *Selective delivery of herpes virus vectors to experimental brain tumors using RMP-7*, Cancer Gene Ther. 6(1):14-20 [1999]).

The calcium-activated potassium channel (K_{Ca}) is an important regulator of cerebral blood vessel tone (Nelson MT, Quayle JM. *Physiological roles and properties of potassium channels in arterial smooth muscle*, Am. J. Physiol. 268(4 Pt 1): C799-822[1995]). The K_{Ca} channel is ubiquitously distributed in tissues as α and β subunits. Its activity is triggered by depolarization and enhanced by an increase in cytosolic calcium di-cation (Ca^{2+}). A local increase in Ca^{2+} is sensed by the extremely sensitive brain α-subunit of the K_{Ca}, directed towards the cytoplasm in the cell, that allows a significant potassium cation flux through these channels.

Under conditions when intracellular cyclic 3', 5' adenosine monophosphate (cAMP) concentration rises in vascular endothelium (e.g. hypoxia), ATP-sensitive potassium channels (K_{ATP}) may also play a role. (J.E. Brian *et al.*, *Recent insights into the regulation of cerebral circulation*, Clin. Exp. Pharmacol. Physiol. 23(6-7):449-57 [1996]). Minoxidil sulfate and chromakalim are reported to be activators of K_{ATP}. (A.D. Wickenden *et al.*, *Comparison of the effects of the K(+)-channel openers cromakalim and minoxidil sulphate on vascular smooth muscle*, Br. J. Pharmacol, 103(1):1148-52 [1991]).

Treatments directed to the use of potassium channel activators or agonists have been taught for disorders including hypertension, cardiac and cerebral ischemia, nicotine addiction, bronchial constriction, and neurodegenerative diseases, but not particularly for the treatment of malignant tumors. (Erhardt *et al.*, *Potassium channel activators/openers*, U.S. Patent No. 5,416,097; Schohe-Loop *et al.*, *4, 4'-bridged bis-2, 4-diaminoquinazolines*, U.S. Patent No. 5,760,230; Sit *et al.*, *4-aryl-3-hydroxyquinolin-2-one derivatives as ion channel modulators*, U.S. Patent No. 5,922,735;Garcia *et al.*, *Biologically active compounds*, U.S. Patent No. 5,399,587; Cherksey, *Potassium channel activating compounds and methods of use thereof*, U.S. Patent No. 5,234,947).

Bradykinin is thought to increase $[Ca^{2+}]_i$ and thus may activate K_{Ca} channels. While some other known activators of K_{Ca} do not act as vasodilators, for example, 1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS-1619; M. Holland *et al.*, *Effects of the BKCa channel activator, NS1619, on rat cerebral artery smooth muscle*, Br J Pharmacol, 117(1):119-29 [1996]), evidence is accumulating that K_{Ca} may play an important role in vasodilatation mediated by vasodilators, such as bradykinin, NO-donors, cGMP, and guanylate cyclase activators. (Berg T., Koteng O., *Signaling pathways in bradykinin- and nitric oxide-induced hypotension in the normotensive rat; role of K+-channels*, Br. J. Pharmacol.;121(6):1113-20 [1997]; Bolotina, V.M. *et al.*, *Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle*, Nature 368(6474):850-3 [1994]; Robertson, B.E., *et al.*, *cGMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells*, Am. J. Physiol. 265(1 Pt 1):C299-303 [1993]; Sobey, C.G. *et al.*, *Mechanisms of bradykinin-induced cerebral vasodilatation in rats. Evidence that reactive oxygen species activate K+ channels*, Stroke 28(11):2290-4; discussion 2295 [1997]; C.G. Sobey and F.M. Faraci, *Effect of nitric oxide and potassium channel agonists and inhibitors on basilar artery diameter*, Am. J. Physiol. 272(1 Pt 2):H256-62 [1997]).

Bradykinin's action as a powerful vasodilator is disadvantageous when using bradykinin to open the blood-brain barrier to therapeutic anticancer agents. Bradykinin or its analogs may adversely lower blood pressure, reduce cerebral blood flow, or contribute to brain edema in some patients. (E.g., A.M. Butt, *Effect of inflammatory agents on electrical resistance across the*

blood-brain barrier in pial microvessels of anesthetized rats, Brain Res. 696(1-2):145-50 [1995]). In addition, bradykinin constricts smooth muscle and stimulates pain receptors.

Consequently, there is still a definite need to maximize the effectiveness of a wide variety of therapeutic agents through enhanced selective transvascular delivery to malignant tumors, including those of the central nervous system, and/or to other abnormal brain regions. These and other benefits the present invention, employing potassium channel agonists, provides as described herein.

SUMMARY OF THE INVENTION

The present invention relates to a method of delivering a medicant to an abnormal brain region in a mammalian subject, including a human. The method includes administering to the subject a potassium channel agonist other than bradykinin or a bradykinin analog, for example NS-1619 or minoxidil, under conditions and in an amount sufficient to increase the permeability to the medicant of a capillary or arteriole delivering blood to cells of the abnormal brain region in the subject. Simultaneously or substantially simultaneously with the potassium channel agonist, the medicant is administered, so that the medicant is delivered selectively to the cells of the abnormal region compared to normal brain regions, due to the increased permeability of capillaries and arterioles supplying the abnormal brain region. The method is particularly valuable in the treatment of physical or biochemical brain injuries caused by trauma, infection, stroke, ischemia, and, particularly, malignant brain tumors, for which survival rates are notoriously poor.

The present invention also relates to a method of delivering a medicant to a malignant tumor in the brain or anywhere in the body of a mammalian subject. The method involves administering to the subject a potassium channel agonist, other than bradykinin or a bradykinin analog, under conditions and in an amount sufficient to increase the permeability to the medicant of a capillary or arteriole delivering blood to cells of the malignant tumor in the subject. Simultaneously or substantially simultaneously with the potassium channel agonist the medicant is administered to the subject, and it is delivered selectively to the malignant cells compared to non-malignant cells by virtue of the potassium channel agonist. The inventive method is useful

in treating any kind of malignant tumor by increasing the selectivity of drug delivery to neoplastic tissue, thereby minimizing damage to non-malignant tissue from medicants, including cytotoxic chemotherapeutic agents, and focusing the therapeutic or diagnostic action of the agents. Thus, this invention, also directed to a method of treating a malignant tumor in a human subject, offers enhanced prospects of survival to cancer patients, with fewer harmful side effects.

The selectivity of the methods is based on the role of calcium- and ATP-dependent potassium transporters (channels) in mediating the permeability of microvasculature to various drugs, macromolecules, and viral particles, combined with the greater number of calcium- and ATP-dependent potassium channels present in abnormal brain vasculature or tumor neomicrovasculature compared to normal microvasculature.

The present invention also relates to a pharmaceutical composition that comprises a combination of a potassium channel agonist, other than bradykinin or a bradykinin analog, formulated in a pharmaceutically acceptable solution together with a medicant for delivery by intravascular infusion or bolus injection into a mammal, such as a human. The pharmaceutical composition is useful in practicing the inventive methods.

The invention also relates to a kit for enhancing the delivery of a medicant to an abnormal brain region and/or to a malignant tumor.

These and other advantages and features of the present invention will be described more fully in a detailed description of the preferred embodiments which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Figure 1A shows the enhancing effect of NS-1619 on blood-tumor barrier permeability to [^{14}C]α-aminoisobutyric acid (AIB) tracer (left) compared to the effect on blood-brain barrier permeability in normal brain tissue adjacent (middle) and contralateral (right) to malignant RG2 glioma tissue in Wistar rats.

Figure 1B shows the enhancing effect of minoxidil sulfate on blood-tumor barrier permeability to [^{14}C]AIB tracer (left) compared to the effect on blood-brain barrier permeability in normal brain tissue adjacent (middle) and contralateral (right) to malignant RG2 glioma tissue in Wistar rats.

Figure 2 shows a dose-response to NS-1619 in the unidirectional transfer constant K_i for [^{14}C]α-aminoisobutyric acid in malignant RG2 glioma tissue in Wistar rats. $K_i = \mu L/g/min$.

Figure 3 shows specific inhibition by iberiotoxin (IBTX; 2.3 $\mu g\ kg^{-1}\ min^{-1}$) of the permeability increasing effect of NS-1619 (26.5 $\mu g\ kg^{-1}\ min^{-1}$). The K_i was determined in RG2 tumor-bearing Wistar rats using [^{14}C]AIB with NS-1619 (26.5 $\mu g\ kg^{-1}\ min^{-1}$) with or without IBTX (2.3 $\mu g\ kg^{-1}\ min^{-1}$), for 15 minutes. The results are compared with PBS, pH 7.4 with or without 5% ethanol.

Figure 4 shows intense over-expression of K_{Ca} as indicated by anti-K_{Ca} immunostain of glioma tissue (Fig. 4B), compared to normal contralateral brain tissue (Fig. 4A). Magnification is 100x.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The inventive methods are useful for selectively delivering a medicant to abnormal brain regions and/or malignant tumors in mammalian subjects. The methods involve administering to the mammalian subject a potassium channel agonist, other than bradykinin or a bradykinin analog, under conditions and in an amount sufficient to increase the permeability to the medicant of a capillary or arteriole delivering blood to cells of the abnormal brain region and/or to malignant cells of a malignant tumor present in the subject. The increase in permeability ranges from at least 2-to 6-fold, compared to controls without the administration of a potassium channel agonist. The relative increase in permeability tends to be greater for large molecular weight medicants (e.g., about 10,000 to 250,000 D) than for smaller molecular weight substances (e.g., about 50-200 D).

The abnormal brain regions include regions of brain tissue physiologically directly affected by a physical or biochemical injury, for example, Alzheimer's disease, Parkinsonism,

trauma, infection, stroke, brain ischemia, or regions of neoplastic growth within the brain, such as benign or malignant brain tumor tissues.

The present invention is also useful for selectively delivering a medicant to a malignant tumor in the brain or to a tumor elsewhere in the body of a mammalian subject. The inventive technology is useful in the treatment of all kinds of solid malignant tumors, including gliomas, glioblastomas, oligodendrogliomas, astrocytomas, ependymomas, primitive neuroectodermal tumors, atypical meningiomas, malignant meningiomas, neuroblastomas, sarcomas, melanomas, lymphomas, or carcinomas. The tumor to be treated can be contained in the skull, brain, spine, thorax, lung, peritoneum, prostate, ovary, uterus, breast, stomach, liver, bowel, colon, rectum, bone, lymphatic system, skin, or in any other organ or tissue of the subject.

The inventive methods are useful in treating any mammal, including a human, non-human primate, canine, feline, bovine, porcine or ovine mammal, as well as in a small mammal such as a mouse, rat, gerbil, hamster, or rabbit.

The potassium channel agonist is an activator of either a calcium-activated potassium channel (K_{Ca}) of any conductance level, whether of large, intermediate, or small conductance, or of an ATP-sensitive potassium channel (K_{ATP}). Examples of useful potassium channel agonists that are K_{Ca} activators include 1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS-1619) or 1-ethyl-2-benzimidazolinone (1-EBIO). Other examples include soluble guanylyl cyclase activators, such as, metalloporphyrins (e.g., zinc or tin protoporphyrin IX), YC-1 (a benzyl indazole derivative), or guanylyl cyclase activating proteins (GCAPs).

Examples of useful potassium channel agonists that are K_{ATP} activators include minoxidil (2,4-diamino-6-piperidino pyramidine-3-oxide; insoluble in water, soluble in ethanol 29 mg/mL), pinacidil ((+/-)-N-cyano-N'-4-pyridinyl-N"-(1,2,2-trimethyl propyl)-guanidine; insoluble in water, soluble in ethanol 14 mg/mL]), (+)-cromakalim, (-)-cromakalim or levcromakalim, (+/-)-cromakalim, or diazoxide. Included among useful potassium channel agonists are pharmaceutically acceptable molecular conjugates or salt forms that still have activity as potassium channel agonists. An example is minoxidil sulfate, but other pharmaceutically acceptable salts comprise anions other than sulfate, such as carbonate, bicarbonate, nitrate, or the

like. Other embodiments of pharmaceutically acceptable salts contain cations, such as sodium, potassium, magnesium, calcium, or the like. Other embodiments of useful potassium channel agonists are hydrochloride salts.

However, the potassium channel agonist employed in the inventive methods is one other than the vasodilator bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), or a polypeptide bradykinin analog, such as receptor mediated permeabilizer (RMP)-7 or A7 (e.g., Kozarich *et al.*, U.S. Patent No. 5,268,164 and PCT Application No. WO 92/18529). Other analogs of bradykinin include related peptide structures which exhibit the same properties as bradykinin but have modified amino acids or peptide extensions on either terminal end of the peptide. Examples of bradykinin analogs include [phe.sup.8 (CH.sub.2 –NH) Arg.sup.9]-bradykinin, N–acetyl [phe.sup.8 (CH.sub.2 --NH--Arg.sup.9] bradykinin and desArg9-bradykinin.

In accordance with the inventive methods, the potassium channel agonist is administered by intravenous or intra-arterial injection or infusion. For treating an abnormal brain region, such as an intracranial tumor, the potassium channel agonist is preferably administered by intracarotid infusion. The amount of potassium channel agonist to be administered ranges from 0.075 to 1500 micrograms per kilogram body mass. For humans the range of 0.075 to 150 micrograms per kilogram body mass is most preferred. This can be administered in a bolus injection, but is preferably administered by infusion over a period of one to thirty minutes, and most preferably during a period of one to fifteen minutes. For example, in rats, a dose rate of about 0.75 to about 100 $\mu g\ kg^{-1}\ min^{-1}$ is most suitable. At dose rates above about 100 $\mu g\ kg^{-1}\ min^{-1}$ a concomitant fall in blood pressure has been observed In humans, effective dose rates are about 0.075 to about 15 $\mu g\ kg^{-1}\ min^{-1}$, with cautious monitoring of blood pressure being advised. The practitioner skilled in the art is also cautious in regulating the total infusion volume, rate of liquid infusion, and electrolyte balance to avoid adverse physiological effects related to these.

Some potassium channel agonists, such as NS-1619, minoxidil, minoxidil sulfate, pinacidil, or diazoxide are not easily dissolved in water; in preparing these agents for administration, a suitable and pharmaceutically acceptable solvent, such as ethanol, can be used to dissolve the potassium channel agonist prior to further dilution with an infusion buffer. The skilled practitioner is cautious in regulating the final concentration of solvent in the infusion

solution to avoid solvent-related toxicity. For example, a final ethanol concentration in an infusion solution up to 5-10% (v/v) is tolerated by most mammalian subjects with negligible toxicity.

While the inventive method does not depend on any particular mechanism by which increased microvascular permeability to the medicant is achieved, it is thought that administration of the potassium channel agonist increases potassium flux through potassium channels in endothelial cell membranes of the capillaries and arterioles delivering blood to abnormal brain regions and/or malignant tumors. This results in a loosening of tight junctions in the microvascular epithelium and/or increased pinocytotic activity, enhancing the uptake of medicants from the blood vessels. In practicing the inventive methods, it is not necessary to measure potassium channel activity (i.e., potassium cation flux therethrough). But the skilled artisan is aware that potassium flux can be measured by any suitable method, for example, by measuring cellular uptake of $^{42}K^+$ or $^{201}Tl^+$ or channel conductance using patch-clamp or microelectrode devices. (e.g., T. Brismar *et al.*, *Thallium-201 uptake relates to membrane potential and potassium permeability in human glioma cells*, Brain Res. 500(1-2):30-36 [1989]; T. Brismar *et al.*, *Mechanism of high K^+ and Tl^+ uptake in cultured human glioma cells*, Cell Mol. Neurobiol. 15(3):351-60 [1995]; S. Cai *et al.*, *Single-channel characterization of the pharmacological properties of the K(Ca2+) channel of intermediate conductance in bovine aortic endothelial cells*, J. Membr. Biol. 163(2):147-58 [1998]).

The medicant is administered simultaneously or substantially simultaneously with the potassium channel agonist, and the medicant is delivered by the blood stream selectively to the abnormal brain region and/or to the malignant cells compared to normal brain tissue or non-malignant cells. "Simultaneously" means that the medicant is administered contemporaneously or concurrently with the potassium channel agonist. "Substantially simultaneously" means that the medicant is administered within about one hour after the potassium channel agonist is last administered, preferably within about 30 minutes after, and most preferably, is administered simultaneously with the potassium channel agonist. Alternatively, "substantially simultaneously" means that the medicant is administered within about 30 minutes before, and preferably within about 15 minutes before the potassium channel agonist is first administered.

The methods of delivering a medicant to an abnormal brain region and/or to a malignant tumor in a mammalian subject are effective in selectively delivering any medicant across the microvascular of an abnormal brain region and/or malignant tumor. The medicant is a drug, i.e., a chemotherapeutic agent. Example of chemotherapeutic agents including therapeutic cytotoxic agents (e.g., cisplatin, carboplatin, methotrexate, 5-fluorouracil, amphotericin), "naked" DNA expression vectors, therapeutic proteins, therapeutic oligonucleotides or nucleotide analogs, interferons, cytokines, or cytokine agonists or antagonists, adrenergic agents, anticonvulsants, anti-trauma agents, or any neuropharmaceutical agent used to treat or prevent an injury or disorder of the brain. Chemotherapeutic agents also include ischemia-protective drugs such as N-methyl-D-aspartate (NMDA) receptor antagonists; antimicrobial agents, such as antibiotics; immunotoxins, immunosuppressants, boron compounds, monoclonal antibodies and specific antigen-binding antibody fragments (e.g., Fab, Fab', $F(ab')_2$, or F(v) fragments), and cytokines, such as interferons, interleukins (e.g., interleukin [IL]-2), tumor necrosis factor (TNF)-α, or transforming growth factors (e.g., TGF-β).

The medicant also includes anticancer chemotherapeutic agents. Typically, anticancer chemotherapeutic agents are cytotoxic agents, such as 5-fluorouracil, cisplatin, carboplatin, methotrexate, daunorubicin, doxorubicin, vincristine, vinblastine, or a cytotoxic alkylating agent, such as, but not limited to, busulfan (1,4-butanediol dimethanesulphonate; Myleran, Glaxo Wellcome), chlorambucil, cyclophosphamide, melphalan, or ethyl ethanesulfonic acid. The anticancer chemotherapeutic agents are particularly useful in practicing the method of selectively delivering a medicant to a malignant tumor, in the brain or in any other tissue of the body, and in the method of treating a malignant tumor in a human subject.

Medicants also include any therapeutic viral particle, for example an adenovirus-derived or herpes simplex virus (HSV)-derived viral vector for delivering genetic material to a cellular target in vivo. Medicants also include diagnostic agents, such as imaging or contrast agents, for example, radioactively labeled substances (e.g., [^{99}Tc]-glucoheptonate), gallium-labeled imaging agents (e.g., gallium-EDTA), ferrous magnetic, fluorescent, luminescent, or iodinated contrast agents. Where suitable, any of the afore-mentioned medicants having anticancer

activity can also be used in practicing the method of selectively delivering a medicant to a malignant tumor or the method of treating a malignant tumor in a human subject.

Thus, the medicant can be a molecular substance having a molecular weight between about 50 D and about 250 kD. Or it can be a particle, such a s a viral particle, having a diameter between about 50 to 250 nanometers.

This is by no means intended to be an exhaustive list of the kinds of medicants that can be employed in practicing the inventive methods. The medicant can be, but is preferably not, an agent that is highly lipid soluble and thus inherently able to penetrate cell membranes, for example nitrosourea.

The amount of medicant that is employed is within a conventional dose range for each medicant, however by practicing the inventive method, the increased transvascular permeability afforded can provide a greater selective therapeutic effect per dose or permit a lower effective dose to be used, if desired, for example to lessen systemic toxic effects from anti-cancer medication in a particular subject.

The medicant is administered by any appropriate method that can deliver it to the blood stream. Typically, this is by intravenous, intramuscular, or intra-arterial (including intracarotid) injection or infusion. However, for some applications other acceptable delivery routes can be used as long as the dose of medicant enters the blood stream substantially simultaneously with the potassium channel agonist. Examples include ingestion (e.g., of a powder, suspension, solution, emulsion, tablet, capsule or caplet); subcutaneous injection; stereotactic injection; or transdermal or transmucosal delivery by adhesive patch, suppository or gel for delivery through the skin, mucosa or epithelium of the mouth including the sublingual epithelium, the rectum, or the vaginal epithelium.

Alternatively, the medicant is administered together with the potassium channel agonist in a pharmaceutical composition of the present invention. The inventive pharmaceutical composition comprises a combination of a potassium channel agonist, other than bradykinin or a bradykinin analog, as described above, formulated in a pharmaceutically acceptable solution together with a medicant, as described above, for delivery by intravascular infusion or bolus injection into a mammal, such as a human. The solution is thus suitably balanced, osmotically

(e.g., about 0.15 M saline) and with respect to pH, typically between pH 7.2 and 7.5; preferably the solution further comprises a buffer, such as a phosphate buffer (e.g., in a phosphate buffered saline solution). The solution is formulated to deliver a dose rate of about 0.075 to 1500 micrograms of potassium channel agonist per kilogram body mass in a pharmaceutically acceptable fluid volume over a maximum of about thirty minutes. For human subjects, the solution is preferably formulated to deliver a dose rate of about 0.075 to 150 micrograms of potassium channel agonist per kilogram body mass in a pharmaceutically acceptable fluid volume over a period of up to about thirty minutes.

The invention also relates to a kit for enhancing the delivery of a medicant to an abnormal brain region and/or to a malignant tumor. The kit is an assemblage of materials or components, including a potassium channel agonist, other than bradykinin or a bradykinin analog, as described above. In addition, the kit contains instructions for using the potassium channel agonist to enhance the permeability of abnormal microvascular, including neomicrovasculature, to a medicant in general, or alternatively, to a particular medicant. Optionally, the kit also contains other components, such as a particular medicant in any pharmaceutically acceptable formulation, or paraphernalia for injection or infusion, for example syringes, infusion lines, clamps, and/or infusion bags/bottles, which can contain a pharmaceutically acceptable infusible formulation of the potassium channel agonist with or without a particular medicant also contained therein. The materials or components assembled in the kit can be provided to the practitioner stored in any convenient and suitable ways that preserve their operability and utility. For example the components can be in dissolved, dehydrated, or lyophilized form; they can be provided at room, refrigerated or frozen temperatures.

The foregoing descriptions of the methods and kits of the present invention are illustrative and by no means exhaustive. The invention will now be described in greater detail by reference to the following non-limiting examples.

EXAMPLES

Example 1: Methods

Malignant Cell Line and Tumor Implantation. A rat glioma cell line, RG2, was used for implantation of experimental brain tumors in Wistar rats. The techniques for RG2 cell propagation and maintenance in tissue culture have been described (Sugita, M. and Black, K.L., *Cyclic GMP-specific phosphodiesterase inhibition and intracarotid bradykinin infusion enhances permeability into brain tumors*, Cancer Res. 58(5):914-20 [1998]; Inamura *et al.* [1994]; Nakano, S. *et al.*, *Increased brain tumor microvessel permeability after intracarotid bradykinin infusion is mediated by nitric oxide*, Cancer Res. 56(17):4027-31 [1996]). Briefly, RG2 cells derived from a rat glioma are kept frozen until use, then are thawed and maintained in a monolayer culture in F12 medium with 10% calf serum.

The Wistar rats (approximately 140-160 g body weight) were anesthetized with intraperitoneal ketamine (50 mg/kg), and glial cells (1×10^5) were implanted into the right hemisphere, but not the contralateral hemisphere, by intracerebral injection suspended in 5 μL F12 medium (1-2% methylcellulose) by a Hamilton syringe. The implantation coordinates were 3-mm lateral to the bregma and 4.5 mm deep to the dural surface.

Intracarotid Infusion of Potassium Channel Activators. Seven days after implantation of RG2 cells, the rats were anesthetized as described above and prepared for permeability studies. Animals were infused with either NS-1619 (a selective large conductance Ca^{2+}- activated K^+ channel activator; RBI, Natick, MA) or minoxidil sulfate (a K_{ATP} channel activator) into the right carotid artery at a dose rate of 7.5 μg kg^{-1} min^{-1} (in 53.3 μL/min) for 15 minutes, in an infusion vehicle of PBS, pH 7.4; 5% (v/v) ethanol. Ethanol (25% [v/v]) was used to dissolve the potassium channel agonists before dilution in PBS. For blood volume studies, 5 and 14 minutes after the start of the intracarotid infusion of potassium channel agonist compounds, [^{14}C] Dextran (100 μCi/kg; Dupont-New England Nuclear Co., Boston, MA) was injected as an intravenous bolus and maintained for 1 minute and 10 minutes to obtain two different time points. For regional permeability studies, 5 minutes after the start of the intracarotid infusion of vasoactive compounds, 100 μCi/kg of [^{14}C]α-aminoisobutyric acid (Dupont-New England Nuclear Co., Boston, MA) was injected as an intravenous bolus. A peristaltic withdrawal pump was used to withdraw femoral arterial blood at a constant rate of 0.083 mL/min immediately after the

injection of the tracer to determine serum radioactivity. Fifteen minutes after the intracarotid infusion, rat decapitated and the brain rapidly removed and frozen for quantitative autoradiography.

Unidirectional Transport Constant (K_i). The unidirectional transfer constant K_i for [^{14}C]α-aminoisobutyric acid was measured in normal tissue and tumor tissue as an indicator of permeability across the blood-tumor and blood-brain barriers. Quantitative autoradiography was used to obtain K_i values ($\mu L\ g^{-1}min^{-1}$). The initial rate for blood-to-brain transfer was calculated using a previously described equation. (Ohno, K, *et al.*, *Lower limits of cerebrovascular permeability to nonelectrolytes in the conscious rat*, Am. J. Physiol. 235(3):H299-307, [1978]; Inamura, T., *et al.*, *Bradykinin selectively opens blood-tumor barrier in experimental brain tumors*, J. Cereb. Flow Metab. 14(5):862-70 [1994]). Quantitative data were analyzed using, two group t-test and two-group Fisher's-exact test of equal proportions or equal means (equal numbers) at 90% power requires a minimum of 6 animals in each group to achieve statistical significance. Multiple treatment groups were compared with control group by ANOVA and P values determined by post-hoc Bonferroni test.

Dose-dependence studies. NS-1619 was dissolved in 25% ethanol and diluted with PBS to obtain various concentrations for infusion. NS-1619 was administered by intracarotid infusion (dose rates: 0, 13, 26.5, 53, 80, 100 and 110 $\mu g\ kg^{-1}\ min^{-1}$; all at 53.3 μL/min) to RG2 glioma-bearing rats to determine a dose that produces increased permeability (K_i) of [^{14}C]-AIB, which was administered intravenously. K_i determined as described above. Physiological parameters were monitored during the experiments.

Inhibition studies. Since NS-16129 increased permeability, the specificity of its effect was examined using the specific K_{Ca} channel inhibitor, iberiotoxin (RBI, Natick, MA). Iberiotoxin (IBTX) was diluted in saline to a final concentration of 100 μg/mL. IBTX (2.3 $\mu g\ kg^{-1}\ min^{-1}$) was co-infused with NS-1619 (26.5 $\mu g\ kg^{-1}\ min^{-1}$) to block K_{Ca} channel-induced permeability in abnormal capillaries in the RG2 glioma model. Seventeen rats were used for these studies (3

vehicle-only control [i.e., PBS ± 5% (v/v) ethanol]; 3 IBTX; 8 NS-1619; 3 NS-1619 + IBTX). K_i for [^{14}C]-AIB was determined by quantitative autoradiography as described earlier by Ohno *et al.* (1978).

Immunohistochemical analysis for K_{Ca} channels. Brain sections (12 μm thick) obtained from the permeability studies were incubated with 1:100 dilution of affinity-purified K_{Ca} channel antibody (Alomone Labs, Jerusalem, Israel) for 1 hour, and biotinylated horse anti-mouse immunoglobin (Vector Laboratories, Burlingame, CA) for 30 minutes. After washing 3 times with PBS, the peroxidase sites were visualized using an avidin:biotinylated enzyme complex (ABC) kit.

Example 2: Results

Potassium Channel Activators Selectively Increase Transport Across the Blood-tumor Barrier.

When Wistar rats bearing implanted glioma cells were infused with either NS-1619 or minoxidil sulfate, at 7.5 $\mu g\ kg^{-1}\ min^{-1}$ for 15 minutes, the unidirectional transport constant K_i for [^{14}C]α-aminoisobutyric acid (AIB) was significantly increased by both NS-1619 and minoxidil sulfate with respect to transport across the neovasculature forming the blood-tumor barrier, but not with respect to transport across normal brain microvasculature. (Figures 1A and 1B). These results demonstrate that activation of potassium calcium channels selectively increases the permeability of molecules across the capillaries of solid malignant tumors compared to capillaries supplying normal brain tissue.

The dose-dependent nature of this increased permeability is demonstrated in Figure 2, which shows that increasing the dose of NS-1619 results in an increase in the unidirectional transfer constant K_i for [^{14}C]α-aminoisobutyric acid in RG2 glioma capillaries. At higher doses (100 and 110 μg/kg/min) a significant drop in the arterial blood pressure of the rats was observed. The numbers of rats used in each group is shown in parentheses in Figures 2.

The specificity of this effect is demonstrated in Figure 3, which shows that the ability of NS-1619 to increase the unidirectional transfer constant K_i for [^{14}C]α-aminoisobutyric acid was

inhibited by the K_{Ca}-channel-specific inhibitor iberiotoxin (IBTX). The K_i was determined in RG2 tumor-bearing rats using [^{14}C] AIB with NS-1619 (26.5 $\mu g\ min^{-1}\ kg^{-1}$)with or without IBTX (2.3 $\mu g\ kg^{-1}\ min^{-1}$; n=3), for 15 minutes. Increase of K_i in response to NS-1619 infusion (n=8; ** P<0.001 compared with PBS with or without 5% ethanol) was attenuated by IBTX co-treatment. IBTX alone at the dose investigated did not affect the brain-tumor barrier permeability of abnormal capillaries. However, IBTX significantly (n=3, ** P<0.001 compared with NS-1619-treated group) decreased NS-1619-induced increase of permeability (K_i), indicating a potassium channel-specific effect. Controls receiving PBS plus 5% ethanol were indistinguishable from controls receiving PBS minus ethanol.

Immunohistochemical Analysis Shows Potassium Channels Are More Abundant in Neovasculature and Malignant Cells Compared to Normal Tissue.

K_{Ca} channel protein was immunolocalized using a specific antibody as described above. Immunohistochemical analysis showed that K_{Ca} channels were selectively increased in tumor tissue and tumor capillaries in RG2 bearing rat brain sections, compared to sections of normal contralateral tissue. (Figure 4). These immunohistochemical results are consistent with the permeability data in which activation of K_{Ca} channel by NS-1619 selectively opened the blood-tumor barrier. (Figure 1A).

Together, the permeability and immunohistochemical data demonstrate that compounds that activate potassium channels can be used to selectively increase delivery of anti-tumor compounds to malignant tumor tissue.

The foregoing examples being illustrative but not an exhaustive description of the embodiments of the present invention, the following claims are presented.

We Claim:

1. A method of delivering a medicant to an abnormal brain region in a mammalian subject, comprising:

administering to a mammalian subject having an abnormal brain region a potassium channel agonist, other than bradykinin or a bradykinin analog, under conditions and in an amount sufficient to increase the permeability to the medicant of a capillary or arteriole delivering blood to cells of the abnormal brain region; and

administering to the subject simultaneously or substantially simultaneously with the potassium channel agonist the medicant, so that the medicant is delivered selectively to the cells of the abnormal brain region compared to normal brain regions.

2. The method of Claim 1, wherein the abnormal brain region is a region of brain tissue physiologically affected by injury, trauma, infection, stroke, or ischemia.

3. The method of Claim 1, wherein the abnormal brain region is a region of benign or malignant tumor tissue.

4. The method of Claim 1, wherein the potassium channel agonist is NS-1619, 1-EBIO, a guanylyl cyclase activator, a guanylyl cyclase activating protein, minoxidil, pinacidil, cromakalim, or levcromakalim.

5. The method of Claim 1, wherein said mammal is a human, non-human primate, canine, feline, bovine, porcine, ovine, mouse, rat, gerbil, hamster, or rabbit.

6. The method of Claim 1, wherein the medicant is a therapeutic cytotoxic agent, DNA expression vector, viral vector, protein, oligonucleotide, nucleotide analog, antimicrobial agent, interferon, cytokine, cytokine agonist, cytokine antagonist, immunotoxin, immunosuppressant, boron compound, monoclonal antibody, adrenergic agent, anticonvulsant, ischemia-protective agent, anti-trauma agent, anticancer chemotherapeutic agent, or diagnostic agent.

7. The method of Claim 6, wherein the diagnostic agent is an imaging or contrast agent.

8. The method of Claim 6, wherein the diagnostic agent is a radioactively labeled substance, a gallium-labeled substance, or a contrast agent selected from the group consisting of ferrous magnetic, fluorescent, luminescent, and iodinated contrast agents.

9. The method of Claim 1, wherein the medicant is a N-methyl-D-aspartate (NMDA) receptor antagonist, antibiotic, interleukin-2; or transforming growth factor-β, cisplatin, carboplatin, tumor necrosis factor-α, methotrexate, 5-fluorouracil, amphotericin, daunorubicin, doxorubicin, vincristine, vinblastine, busulfan, chlorambucil, cyclophosphamide, melphalan, or ethyl ethanesulfonic

acid.

10. The method of Claim 6, wherein the viral vector is an adenovirus-derived vector or herpes simplex virus-derived vector.

11. The method of Claim 1, wherein administering the potassium channel agonist is by intravenous or intra-arterial infusion or injection.

12. The method of Claim 1, wherein administering the potassium channel agonist is by intracarotid infusion or injection.

13. The method of Claim 1, wherein the potassium channel agonist is administered to the mammalian subject by a bolus injection.

14. The method of Claim 1, wherein the potassium channel agonist is administered to the mammalian subject in an amount from about 0.075 to 1500 micrograms per kilogram body mass.

15. The method of Claim 14, wherein the potassium channel agonist is administered to the subject in an amount from about 0.075 to 150 micrograms per kilogram body mass.

16. The method of Claim 1, wherein the potassium channel agonist is administered to the mammalian subject at a dose rate of about 0.075 to about 100 $\mu g\ kg^{-1}\ min^{-1}$ for up to about 30 minutes.

17. The method of Claim 16, wherein the potassium channel agonist is administered to the mammalian subject at a dose rate of about 0.075 to about 15 $\mu g\ kg^{-1}\ min^{-1}$.

18. A method of selectively delivering a medicant to an abnormal brain region in a mammalian subject, comprising:

administering to a mammalian subject having an abnormal brain region a potassium channel agonist, other than bradykinin or a bradykinin analog, under conditions and in an amount sufficient to increase potassium flux through a calcium-activated or ATP-sensitive potassium channel in an endothelial cell membrane of a capillary or arteriole delivering blood to cells of the abnormal brain region, whereby the capillary or arteriole is made more permeable to the medicant; and

administering to the subject simultaneously or substantially simultaneously with the potassium channel agonist the medicant, so that the medicant is delivered selectively to the cells of the abnormal brain region compared to normal brain regions.

19. The method of Claim 18, wherein the abnormal brain region is a region of brain tissue physiologically affected by injury, trauma, infection, stroke, or ischemia.

20. The method of Claim 18, wherein the abnormal brain region is a region of benign or malignant tumor tissue.

21. The method of Claim 18, wherein the potassium channel agonist is NS-1619, 1-EBIO, a guanylyl cyclase activator, a guanylyl cyclase activating protein, minoxidil, pinacidil, cromakalim, or levcromakalim.

22. The method of Claim 18, wherein said mammal is a human, non-human primate, canine, feline, bovine, porcine, ovine, mouse, rat, gerbil, hamster, or rabbit.

23. The method of Claim 18, wherein the medicant is a therapeutic cytotoxic agent, DNA expression vector, viral vector, protein, oligonucleotide, nucleotide analog, antimicrobial agent, interferon, cytokine, cytokine agonist, cytokine antagonist, immunotoxin, immunosuppressant, boron compound, monoclonal antibody, adrenergic agent, anticonvulsant, ischemia-protective agent, anti-trauma agent, anticancer chemotherapeutic agent, or diagnostic agent.

24. The method of Claim 23, wherein the diagnostic agent is an imaging or contrast agent.

25. The method of Claim 23, wherein the diagnostic agent is a radioactively labeled substance, a gallium-labeled substance, or a contrast agent selected from the group consisting of ferrous magnetic, fluorescent, luminescent, and iodinated contrast agents.

26. The method of Claim 18, wherein the medicant is a N-methyl-D-aspartate (NMDA) receptor antagonist, antibiotic, interleukin-2; or transforming growth factor-β, cisplatin, carboplatin, tumor necrosis factor-α, methotrexate, 5-fluorouracil, amphotericin, daunorubicin, doxorubicin, vincristine, vinblastine, busulfan, chlorambucil, cyclophosphamide, melphalan, or ethyl ethanesulfonic acid.

27. The method of Claim 23, wherein the viral vector is an adenovirus-derived vector or herpes simplex virus-derived vector.

28. The method of Claim 18, wherein administering the potassium channel agonist is by intravenous or intra-arterial infusion or injection.

29. The method of Claim 18, wherein administering the potassium channel agonist is by intracarotid infusion or injection.

30. The method of Claim 18, wherein the potassium channel agonist is administered to the mammalian subject by a bolus injection.

31. The method of Claim 18, wherein the potassium channel agonist is administered to the mammalian subject in an amount from about 0.075 to 1500 micrograms per kilogram body mass.

32. The method of Claim 31, wherein the potassium channel agonist is administered to the subject in an amount from about 0.075 to 150 micrograms per kilogram body mass.

33. The method of Claim 18, wherein the potassium channel agonist is administered to the mammalian subject at a dose rate of about 0.075 to about 100 $\mu g\ kg^{-1}\ min^{-1}$ for up to about 30 minutes.

34. The method of Claim 33, wherein the potassium channel agonist is administered to the mammalian subject at a dose rate of about 0.075 to about 15 $\mu g\ kg^{-1}\ min^{-1}$.

35. A method of delivering a medicant to a malignant tumor in a mammalian subject, comprising:

administering to a mammalian subject having a malignant tumor a potassium channel agonist, other than bradykinin or a bradykinin analog, under conditions and in an amount sufficient to increase the permeability to the medicant of a capillary or arteriole delivering blood to cells of the malignant tumor; and

administering to the subject simultaneously or substantially simultaneously with the potassium channel agonist the medicant, so that the medicant is delivered selectively to the malignant cells compared to non-malignant cells.

36. The method of Claim 35, wherein the potassium channel agonist is NS-1619, 1-EBIO, a guanylyl cyclase activator, a guanylyl cyclase activating protein, minoxidil, pinacidil, cromakalim, or levcromakalim.

37. The method of Claim 35, wherein the malignant tumor is a glioma, glioblastoma, oligodendroglioma, astrocytoma, ependymoma, primitive neuroectodermal tumor, atypical meningioma, malignant meningioma, neuroblastoma, sarcoma, melanoma, lymphoma, or carcinoma.

38. The method of Claim 35, wherein the malignant tumor is contained in the skull, brain, spine, thorax, lung, peritoneum, prostate, ovary, uterus, breast, stomach, liver, bowel, colon, rectum, bone, lymphatic system, or skin, of said subject.

39. The method of Claim 35, wherein said mammal is a human, non-human primate, canine, feline, bovine, porcine, ovine, mouse, rat, gerbil, hamster, or rabbit.

40. The method of Claim 35, wherein the medicant is a therapeutic cytotoxic agent, DNA expression vector, viral vector, protein, oligonucleotide, nucleotide analog, antimicrobial agent, interferon, cytokine, cytokine agonist, cytokine antagonist, immunotoxin, immunosuppressant, boron compound, monoclonal antibody, adrenergic agent, anticonvulsant, ischemia-protective agent, anti-trauma agent, anticancer chemotherapeutic agent, or diagnostic agent.

41. The method of Claim 40, wherein the diagnostic agent is an imaging or contrast agent.

42. The method of Claim 40, wherein the diagnostic agent is a radioactively labeled substance, a gallium-labeled substance, or a contrast agent selected from the group consisting of ferrous magnetic, fluorescent, luminescent, and iodinated contrast agents.

43. The method of Claim 35, wherein the medicant is a N–methyl-D-aspartate (NMDA) receptor antagonist, antibiotic, interleukin-2; or transforming growth factor-β, cisplatin, carboplatin, tumor necrosis factor-α, methotrexate, 5-fluorouracil, amphotericin, daunorubicin, doxorubicin,

vincristine, vinblastine, busulfan, chlorambucil, cyclophosphamide, melphalan, or ethyl ethanesulfonic acid.

44. The method of Claim 40, wherein the viral vector is an adenovirus-derived vector or herpes simplex virus-derived vector.

45. The method of Claim 35, wherein administering the potassium channel agonist is by intravenous or intra-arterial infusion or injection.

46. The method of Claim 35, wherein the tumor is an intracranial tumor and the potassium channel agonist is administered by intracarotid infusion or injection.

47. The method of Claim 35, wherein the potassium channel agonist is administered to the mammalian subject by a bolus injection.

48. The method of Claim 35, wherein the potassium channel agonist is administered to the mammalian subject in an amount from about 0.075 to 1500 micrograms per kilogram body mass.

49. The method of Claim 48, wherein the potassium channel agonist is administered to the mammalian subject in an amount from about 0.075 to 150 micrograms per kilogram body mass.

50. The method of Claim 35, wherein the potassium channel agonist is administered to the mammalian subject at a dose rate of about 0.075 to about 100 $\mu g\ kg^{-1}\ min^{-1}$ for up to about 30 minutes.

51. The method of Claim 50, wherein the potassium channel agonist is administered to the mammalian subject at a dose rate of about 0.075 to about 15 $\mu g\ kg^{-1}\ min^{-1}$.

52. A method of delivering a medicant to a malignant tumor in a mammalian subject, comprising:

administering to the mammalian subject having a malignant tumor a potassium channel agonist,

other than bradykinin or a bradykinin analog, under conditions and in an amount sufficient to increase potassium flux through a calcium-activated or ATP-sensitive potassium channel in an endothelial cell membrane of a capillary or arteriole delivering blood to malignant cells of the tumor, whereby the capillary or arteriole is made more permeable to the medicant; and

administering to the subject simultaneously or substantially simultaneously with the potassium channel agonist the medicant, so that the medicant is delivered selectively to the malignant cells compared to non-malignant cells.

53. The method of Claim 52, wherein the potassium channel agonist is NS-1619, 1-EBIO, a guanylyl cyclase activator, a guanylyl cyclase activating protein, minoxidil, pinacidil, cromakalim, or levcromakalim.

54. The method of Claim 52, wherein the malignant tumor is a glioma, glioblastoma, oligodendroglioma, astrocytoma, ependymoma, primitive neuroectodermal tumor, atypical meningioma, malignant meningioma, neuroblastoma, sarcoma, melanoma, lymphoma, or carcinoma.

55. The method of Claim 52, wherein the malignant tumor is contained in the skull, brain, spine, thorax, lung, peritoneum, prostate, ovary, uterus, breast, stomach, liver, bowel, colon, rectum, bone, lymphatic system, or skin, of said subject.

56. The method of Claim 52, wherein said mammal is a human, non-human primate, canine, feline, bovine, porcine, ovine, mouse, rat, gerbil, hamster, or rabbit.

57. The method of Claim 52, wherein the medicant is a therapeutic cytotoxic agent, DNA expression vector, viral vector, protein, oligonucleotide, nucleotide analog, antimicrobial agent, interferon, cytokine, cytokine agonist, cytokine antagonist, immunotoxin, immunosuppressant, boron compound, monoclonal antibody, adrenergic agent, anticonvulsant, ischemia-protective agent, anti-trauma agent, anticancer chemotherapeutic agent, or diagnostic agent.

58. The method of Claim 57, wherein the diagnostic agent is an imaging or contrast agent.

59. The method of Claim 57, wherein the diagnostic agent is a radioactively labeled substance, a gallium-labeled substance, or a contrast agent selected from the group consisting of ferrous magnetic, fluorescent, luminescent, and iodinated contrast agents.

60. The method of Claim 52, wherein the medicant is a N–methyl-D-aspartate (NMDA) receptor antagonist, antibiotic, interleukin-2; or transforming growth factor-β, cisplatin, carboplatin, tumor necrosis factor-α, methotrexate, 5-fluorouracil, amphotericin, daunorubicin, doxorubicin, vincristine, vinblastine, busulfan, chlorambucil, cyclophosphamide, melphalan, or ethyl ethanesulfonic acid.

61. The method of Claim 57, wherein the viral vector is an adenovirus-derived vector or herpes simplex virus-derived vector.

62. The method of Claim 52, wherein administering the potassium channel agonist is by intravenous or intra-arterial infusion or injection.

63. The method of Claim 52, wherein the tumor is an intracranial tumor and the potassium channel agonist is administered by intracarotid infusion or injection.

64. The method of Claim 52, wherein the potassium channel agonist is administered to the mammalian subject by a bolus injection.

65. The method of Claim 52, wherein the potassium channel agonist is administered to the mammalian subject in an amount from about 0.075 to 1500 micrograms per kilogram body mass.

66. The method of Claim 65, wherein the potassium channel agonist is administered to the mammalian subject in an amount from about 0.075 to 150 micrograms per kilogram body mass.

67. The method of Claim 52, wherein the potassium channel agonist is administered to the mammalian subject at a dose rate of about 0.075 to about 100 $\mu g\ kg^{-1}\ min^{-1}$ for up to about 30 minutes.

68. The method of Claim 67, wherein the potassium channel agonist is administered to the mammalian subject at a dose rate of about 0.075 to about 15 $\mu g\ kg^{-1}\ min^{-1}$.

69. A method of treating a malignant tumor in a human subject, comprising:

administering to a human subject having a malignant tumor a potassium channel agonist, other than bradykinin or a bradykinin analog, under conditions and in an amount sufficient to increase the permeability to the medicant of a capillary or arteriole delivering blood to cells of the malignant tumor; and

administering to the subject simultaneously or substantially simultaneously with the potassium channel agonist the medicant, so that the medicant is delivered selectively to the malignant cells compared to non-malignant cells.

70. The method of Claim 69, wherein the potassium channel agonist is NS-1619, 1-EBIO, a guanylyl cyclase activator, a guanylyl cyclase activating protein, minoxidil, pinacidil, cromakalim, or levcromakalim.

71. The method of Claim 69, wherein the malignant tumor is a glioma, glioblastoma, oligodendroglioma, astrocytoma, ependymoma, primitive neuroectodermal tumor, atypical meningioma, malignant meningioma, neuroblastoma, sarcoma, melanoma, lymphoma, or carcinoma.

72. The method of Claim 69, wherein the malignant tumor is contained in the skull, brain, spine, thorax, lung, peritoneum, prostate, ovary, uterus, breast, stomach, liver, bowel, colon, rectum, bone, lymphatic system, or skin, of said subject.

73. The method of Claim 69, wherein the medicant is a therapeutic cytotoxic agent, DNA expression vector, viral vector, protein, oligonucleotide, nucleotide analog, antimicrobial agent, interferon, cytokine, cytokine agonist, cytokine antagonist, immunotoxin, immunosuppressant, boron compound, monoclonal antibody, adrenergic agent, anticonvulsant, ischemia-protective agent, anti-trauma agent, anticancer chemotherapeutic agent, or diagnostic agent.

74. The method of Claim 73, wherein the diagnostic agent is an imaging or contrast agent.

75. The method of Claim 73, wherein the diagnostic agent is a radioactively labeled substance, a gallium-labeled substance, or a contrast agent selected from the group consisting of ferrous magnetic, fluorescent, luminescent, and iodinated contrast agents.

76. The method of Claim 69, wherein the medicant is a N–methyl-D-aspartate (NMDA) receptor antagonist, antibiotic, interleukin-2; or transforming growth factor-β, cisplatin, carboplatin, tumor necrosis factor-α, methotrexate, 5-fluorouracil, amphotericin, daunorubicin, doxorubicin, vincristine, vinblastine, busulfan, chlorambucil, cyclophosphamide, melphalan, or ethyl ethanesulfonic acid.

77. The method of Claim 73, wherein the viral vector is an adenovirus-derived vector or herpes simplex virus-derived vector.

78. The method of Claim 69, wherein administering the potassium channel agonist is by intravenous or intra-arterial infusion or injection.

79. The method of Claim 69, wherein the tumor is an intracranial tumor and the potassium channel agonist is administered by intracarotid infusion.

80. The method of Claim 69, wherein the potassium channel agonist is administered to the mammalian subject by a bolus injection.

81. The method of Claim 69, wherein the potassium channel agonist is administered to the subject in an amount from about 0.075 to 150 micrograms per kilogram body mass.

82. The method of Claim 69, wherein the potassium channel agonist is administered to the subject at a dose rate of about 0.075 to about 15 $\mu g\ kg^{-1}\ min^{-1}$.

83. A method of treating a malignant tumor in a human subject, comprising:

administering to a human subject, having a malignant tumor, a potassium channel agonist, other than bradykinin or a bradykinin analog, under conditions and in an amount sufficient to increase

potassium flux through a calcium-activated or ATP-sensitive potassium channel in an endothelial cell membrane of a capillary or arteriole delivering blood to malignant cells of the malignant tumor, whereby the capillary or arteriole is made more permeable to the medicant; and

administering to the subject simultaneously or substantially simultaneously with the potassium channel agonist the medicant, so that the medicant is delivered selectively to the malignant cells compared to non-malignant cells.

84. The method of Claim 83, wherein the potassium channel agonist is NS-1619, 1-EBIO, a guanylyl cyclase activator, a guanylyl cyclase activating protein, minoxidil, pinacidil, cromakalim, or levcromakalim.

85. The method of Claim 83, wherein the malignant tumor is a glioma, glioblastoma, oligodendroglioma, astrocytoma, ependymoma, primitive neuroectodermal tumor, atypical meningioma, malignant meningioma, neuroblastoma, sarcoma, melanoma, lymphoma, or carcinoma.

86. The method of Claim 83, wherein the malignant tumor is contained in the skull, brain, spine, thorax, lung, peritoneum, prostate, ovary, uterus, breast, stomach, liver, bowel, colon, rectum, bone, lymphatic system, or skin, of said subject.

87. The method of Claim 83, wherein the medicant is a therapeutic cytotoxic agent, DNA expression vector, viral vector, protein, oligonucleotide, nucleotide analog, antimicrobial agent, interferon, cytokine, cytokine agonist, cytokine antagonist, immunotoxin, immunosuppressant, boron compound, monoclonal antibody, adrenergic agent, anticonvulsant, ischemia-protective agent, anti-trauma agent, anticancer chemotherapeutic agent, or diagnostic agent.

88. The method of Claim 87, wherein the diagnostic agent is an imaging or contrast agent.

89. The method of Claim 87, wherein the diagnostic agent is a radioactively labeled substance, a gallium-labeled substance, or a contrast agent selected from the group consisting of ferrous magnetic, fluorescent, luminescent, and iodinated contrast agents.

90. The method of Claim 83, wherein the medicant is a N–methyl-D-aspartate (NMDA) receptor antagonist, antibiotic, interleukin-2; or transforming growth factor-β, cisplatin, carboplatin, tumor necrosis factor-α, methotrexate, 5-fluorouracil, amphotericin, daunorubicin, doxorubicin, vincristine, vinblastine, busulfan, chlorambucil, cyclophosphamide, melphalan, or ethyl ethanesulfonic acid.

91. The method of Claim 87, wherein the viral vector is an adenovirus-derived vector or herpes simplex virus-derived vector.

92. The method of Claim 83, wherein administering the potassium channel agonist is by intravenous or intra-arterial injection.

93. The method of Claim 83, wherein the tumor is an intracranial tumor and the potassium channel agonist is administered by intracarotid infusion.

94. The method of Claim 83, wherein the potassium channel agonist is administered to the mammalian subject by a bolus injection.

95. The method of Claim 83, wherein the potassium channel agonist is administered to the subject in an amount from about 0.075 to 150 micrograms per kilogram body mass.

96. The method of Claim 83, wherein the potassium channel agonist is administered to the mammalian subject at a dose rate of about 0.075 to about 15 $\mu g\ kg^{-1}\ min^{-1}$.

97. A pharmaceutical composition comprising a combination of a potassium channel agonist, other than bradykinin or a bradykinin analog, formulated in a pharmaceutically acceptable solution together with a medicant for delivery by intravascular infusion or injection into a mammal.

98. The pharmaceutical composition of Claim 97, wherein the solution is formulated to deliver a dose rate of about 0.075 to 1500 micrograms per kilogram body mass in a pharmaceutically acceptable fluid volume over a maximum of about thirty minutes.

99. The pharmaceutical composition of Claim 97, wherein the solution is formulated to deliver a dose rate of about 0.075 to 150 micrograms per kilogram body mass in a pharmaceutically acceptable fluid volume over a period of up to thirty minutes.

100. The pharmaceutical composition of Claim 97, wherein the potassium channel agonist is NS-1619, 1-EBIO, a guanylyl cyclase activator, a guanylyl cyclase activating protein, minoxidil, pinacidil, cromakalim, or levcromakalim.

101. The pharmaceutical composition of Claim 97, wherein the medicant is a therapeutic cytotoxic agent, DNA expression vector, viral vector, protein, oligonucleotide, nucleotide analog, antimicrobial agent, interferon, cytokine, cytokine agonist, cytokine antagonist, immunotoxin, immunosuppressant, boron compound, monoclonal antibody, adrenergic agent, anticonvulsant, ischemia-protective agent, anti-trauma agent, anticancer chemotherapeutic agent, or diagnostic agent.

102. The pharmaceutical composition of Claim 101, wherein the diagnostic agent is an imaging or contrast agent.

103. The pharmaceutical composition of Claim 101, wherein the diagnostic agent is a radioactively labeled substance, a gallium-labeled substance, or a contrast agent selected from the group consisting of ferrous magnetic, fluorescent, luminescent, and iodinated contrast agents.

104. The pharmaceutical composition of Claim 97, wherein the medicant is a N–methyl-D-aspartate (NMDA) receptor antagonist, antibiotic, interleukin-2; or transforming growth factor-β, cisplatin, carboplatin, tumor necrosis factor-α, methotrexate, 5-fluorouracil, amphotericin, daunorubicin, doxorubicin, vincristine, vinblastine, busulfan, chlorambucil, cyclophosphamide, melphalan, or ethyl ethanesulfonic acid.

105. The pharmaceutical composition of Claim 101, wherein the viral vector is an adenovirus-derived vector or herpes simplex virus-derived vector.

106. The pharmaceutical composition of Claim 97, further comprising a buffer solution

pharmaceutically acceptable for intravascular infusion into a mammal.

107. The pharmaceutical composition of Claim 106, wherein the buffer solution is phosphate buffered saline.

108. A kit for enhancing the delivery of a medicant to an abnormal brain region and/or to a malignant tumor, comprising:

a potassium channel agonist, other than bradykinin or a bradykinin analog; and

instructions for using the potassium channel agonist for enhancing the delivery of a medicant to an abnormal brain region or to a malignant tumor.

109. The kit of Claim 108, wherein the potassium channel agonist is NS-1619, 1-EBIO, a guanylyl cyclase activator, a guanylyl cyclase activating protein, minoxidil, pinacidil, cromakalim, or levcromakalim.

ABSTRACT OF THE DISCLOSURES

Disclosed are methods of selectively delivering a medicant to an abnormal brain region and/or to a malignant tumor in a mammalian subject, including a human. A medicant is administered simultaneously or substantially simultaneously with a potassium channel agonist (other than bradykinin or a bradykinin analog), such as NS-1619,1-EBIO, a guanylyl cyclase activator, a guanylyl cyclase activating protein, minoxidil, pinacidil, cromakalim, or levcromakalim, whereby the medicant is delivered selectively to the cells of the abnormal brain region and/or to the tumor, compared to normal tissues. Thus, among the disclosures is a method of treating a malignant tumor in a human subject. Also disclosed are pharmaceutical compositions that combine a potassium channel agonist together with a medicant and a kit for enhancing the delivery of a medicant to an abnormal brain region and/or to a malignant tumor.

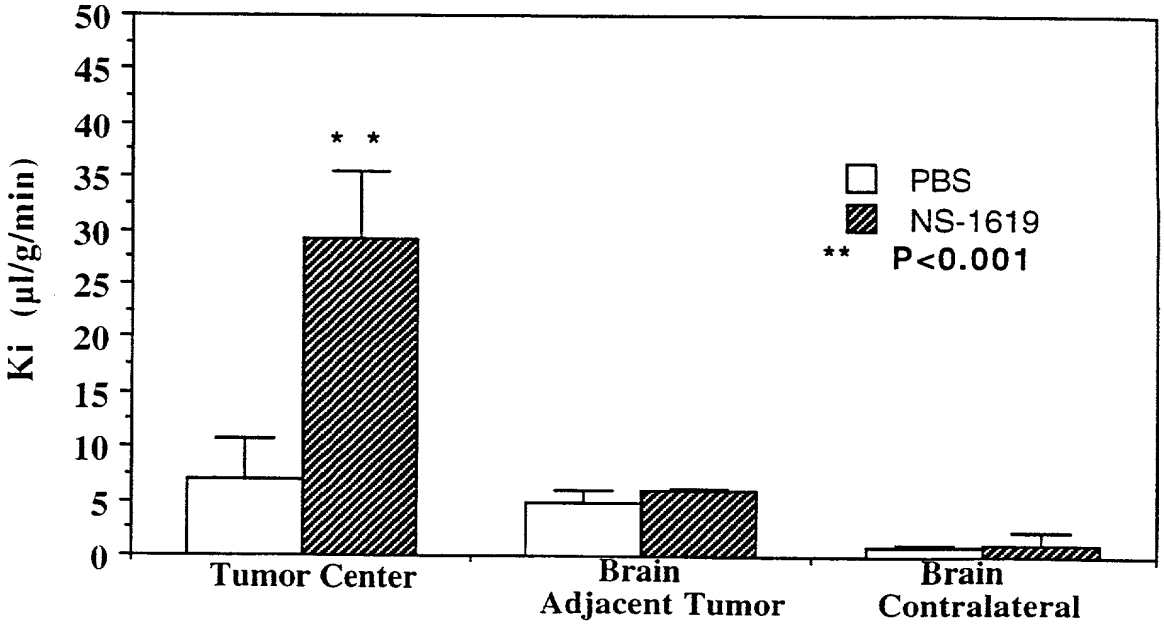


Figure 1A

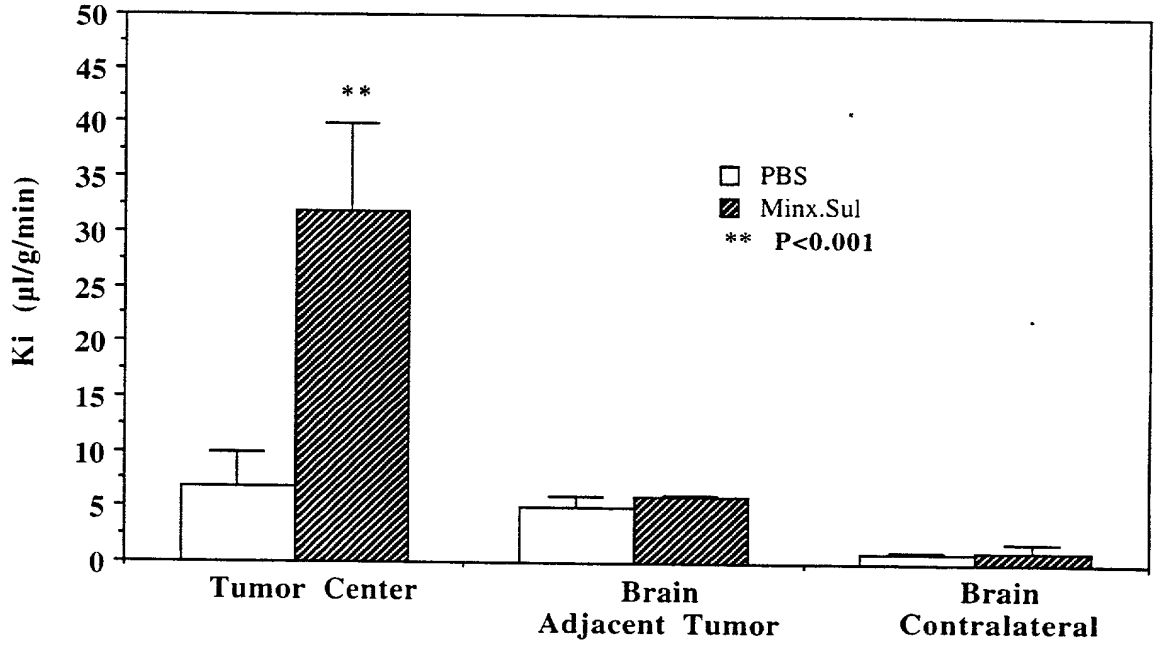


Figure 1B

Figure 2

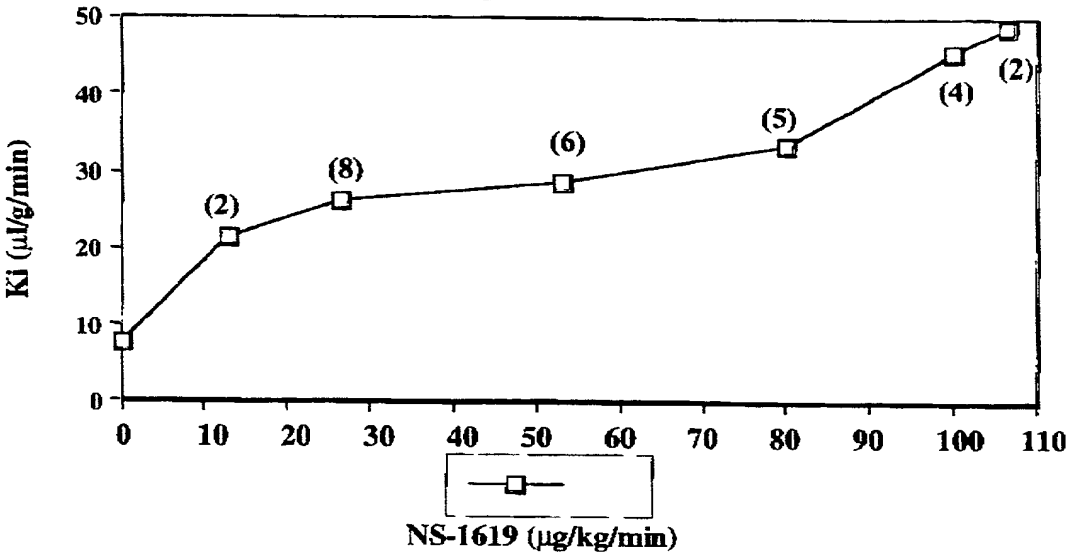
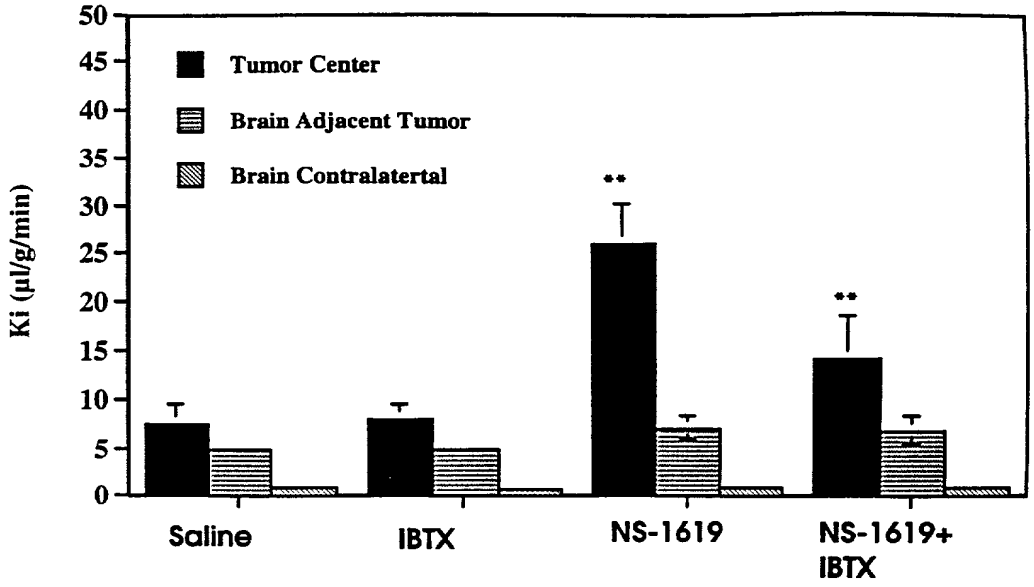


Figure 3



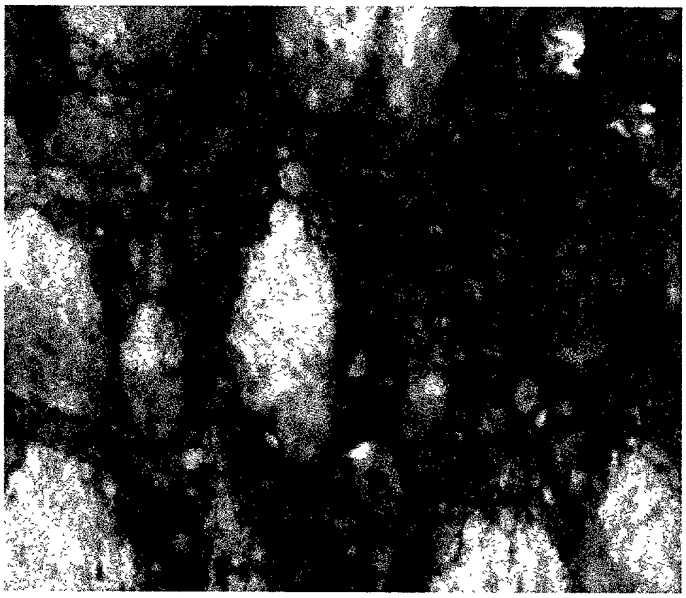


Figure 4A

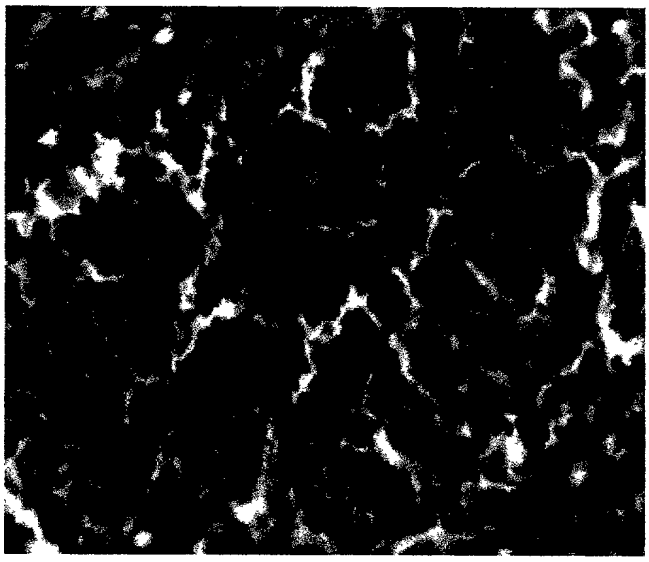


Figure 4B

Docket No.
CEDAR 043453

Declaration For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD FOR USING POTASSIUM CHANNEL AGONISTS FOR DELIVERING A MEDICANT TO AN ABNORMAL BRAIN REGION AND/OR A MALIGNANT TUMOR

the specification of which

(check one)

☒ is attached hereto.

☐ was filed on _____ as United States Application No. or PCT International Application Number _____

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, CFR Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Full name of third inventor, if any
Third inventor's signature — Date
Residence
Citizenship
Post Office Address

Full name of fourth inventor, if any
Fourth inventor's signature — Date
Residence
Citizenship
Post Office Address

CEDAR 043453 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Keith L. Black and Nagendra S. Ningaraj
Serial No. Not Assigned
Filed: Herewith
For: METHOD FOR USING POTASSIUM CHANNEL AGONISTS FOR DELIVERING A MEDICANT TO AN ABNORMAL BRAIN REGION AND/OR A MALIGNANT TUMOR

Examiner: Unassigned
Unit: --

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BOX NEW PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D. C. 20231

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DATE OF DEPOSIT NAME OF PERSON MAKING DEPOSIT

Ann Weiss EL 349 964388 US
SIGNATURE OF PERSON MAKING DEPOSIT EXPRESS MAIL LABEL NUMBER

The undersigned assignee of the entire interest in the above-identified patent application hereby appoints the following attorneys to prosecute and transact all business in the United States Patent and Trademark Office relating to this application: Laurence H. Pretty, Esq., 25,312; Robert A. Schroeder, Esq., 25,373; Edward G. Poplawski, Esq., Reg. No. 33,439; Jeffrey F. Craft, Esq., Reg. No. 30,044; Richard A. Wallen, Esq., Reg. No. 22,671; Michael J. MacDermott, Esq., Reg. No. 29,946; Anne Wang, Esq., Reg. No. 36,045; Paul D. Tripodi, Esq., Reg. No. 40,847; Marc E. Hankin, Esq., Reg. No. 38,908; Donald C. Kordich, Esq. Reg. No. 38,213; J. Christopher James, Esq., Reg. No. 40,660; Daniel C. Mallery, Esq., Reg. No. 33,532; Matthew C. Lapple, Reg. No. 44,203; Carl A. Kukkonen, Reg. No. 42,773; Denise McKenzie, Reg. No. 43,790; Janaki Komanduri, Reg. No. 40, 684; Michael L. Crapenhoft, Esq., Reg. No. 37,115; and Nisan A. Steinberg, Esq., Reg. No. 40,345.

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Respectfully submitted,

CEDARS-SINAI MEDICAL CENTER

Dated: 1/24/00

Peter E. Braveman
Senior Vice-President for
Legal Affairs and General Counsel

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